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Optimizing the Identification of Citrullinated Peptides by Mass Spectrometry

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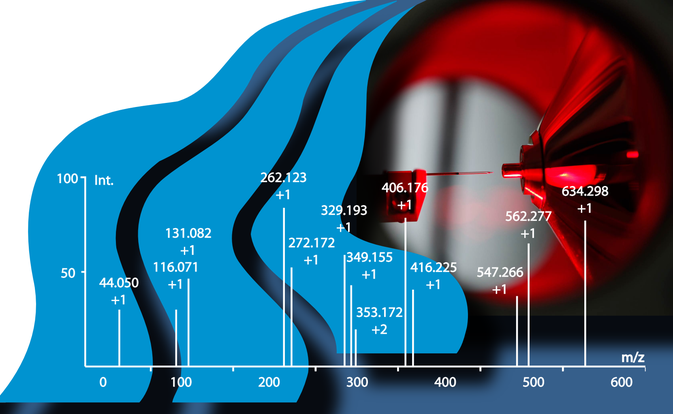
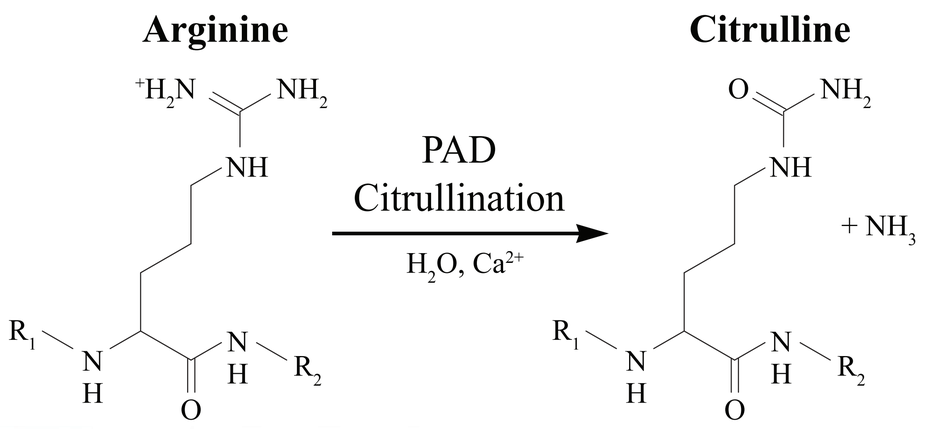


AIM

- Investigate the cleavage properties of trypsin after a citrulline residue.
- Investigate the behavior of citrullinated peptides by reversed phase chromatography.
- Propose a verification strategy for detected citrullinated peptides in a MS workflow.

Introduction

Citrullination is a PAD-enzyme catalyzed deimination of arginine, yielding the non-standard amino acid citrulline.[1]



Protein citrullination has been associated with several diseases and auto-antibodies against citrullinated proteins are today used as an important clinical biomarker in rheumatoid arthritis.[2,7] The site-specific characterization of citrullination using mass spectrometry remains problematic, especially as citrullination and deamidation of asparagine or glutamine results in the same mass increase of +0.984016 Da. The verification, therefore, often relies on a tryptic miscleavage after citrulline.[3] Furthermore, the mass increase is close to that of a neutron, +1.08665 Da.

However, tryptic cleavage after citrulline has in some cases been reported, so we here investigate the cleavage properties of trypsin after a citrulline residue.

Method

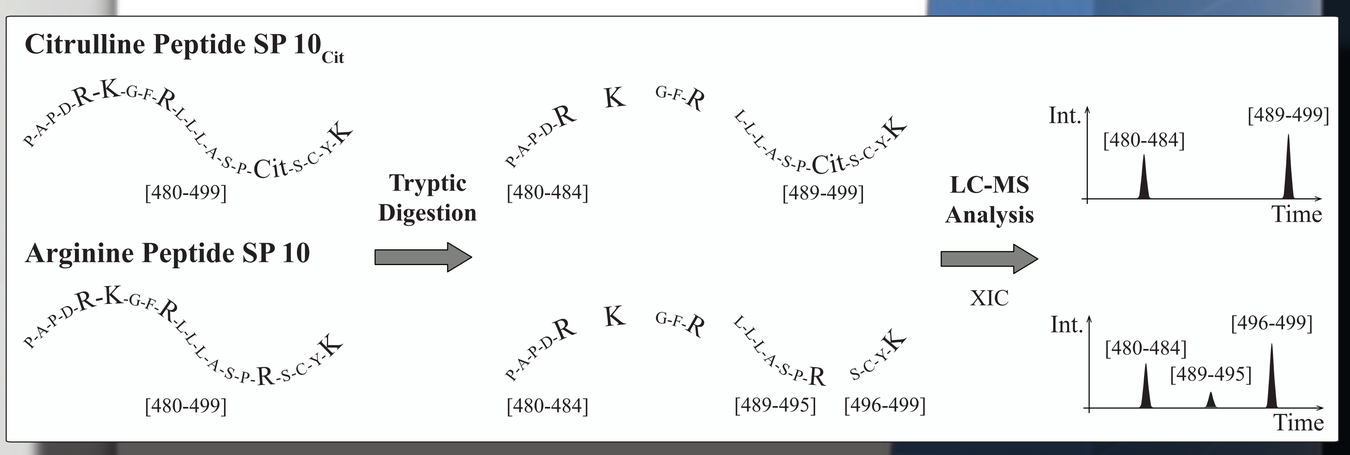
24 synthetic peptide sets containing either arginine or citrulline were analyzed (*JPT Peptide Technologies GmbH*). The peptide sequences originated from disease-associated *in vivo* citrullinated proteins. In-solution tryptic digestion was performed with sequencing grade trypsin (*Promega*). 1 pmol sample was analysed using ESI LC-MS/MS in positive ion mode, on a hybrid microQTOF mass spectrometer (*Bruker*). The peptides were seperated using an in-house packed 10 cm reversed phase C18 column (*Dr. Maisch; reprosil-pur C18-AQ*) with acetonitrile.

Extracted ion chromatograms (XIC) were constructed in Bruker Daltonics DataAnalysis v 3.4, with all predicted tryptic peptides +/- m/z 0.01, under the assumption that trypsin cleaves after arginine, lysine and citrulline.

SP #	Sequence	Protein ID	% RT shift
1	DS **R/Cit** GNPTVEVDLFTS**K**GLF**R**	P06733.2	2.4
2	DPS **R/Cit** YISPDQLADLY**K**SFI**K**	P06733.2	3.0
3	EELGS**K**A**K**FAG **R/Cit** NF **R/Cit** NPLA**K**	P06733.2	1.6
4	VTTST**R**TYSLGSAL **R/Cit** PSTS**R**	AAH66956	1.4
5	EQL**K**GQG**K**S **R/Cit** LGDLYEEEM**R**	AAH66956	2.6
6	NM**K**EEMA**R**HL **R/Cit** EYQDLLNV**K**	AAH66956	1.4
7	NM**K**EEMA **R/Cit** HL**R**EYQDLLNV**K**	AAH66956	2.0
8	LHVA **R/Cit** SEMD**K**V **R/Cit** VFQAT **R/Cit** G**K**	NP 036519.2	3.6
9	GL**K**EFPI**K** **R/Cit** VMGPDFGYVT**R**	NP 036519	3.4
10	PAPD**R**K**G**F**R**LLLASP **R/Cit** SCY**K**	NP 036519	2.0
11	LS **R/Cit** TV**R**CTCISISNQPVNP**R**	P02778.2	1.6
12	EMHG**K**NWS**K**LC **R/Cit** DCQVIDG**R**	ACB10579	1.6
13	SGVT**K**AISSPTVS **R/Cit** LTDTT**K**	ACB10579	1.4
14	AEGGGV **R/Cit** GP**R**VVE **R/Cit** HQSAC**K**	P02671	0
15	SHHPGIAEFPS **R/Cit** G**K**SSSYS**K**	P02671	0.4
16	FTSSTSYN **R/Cit** GDSTFES**K**SY**K**	P02671	1.0
17	A **R/Cit** HGFLP **R/Cit** H**R**DTGILDSIG**R**	P02686.3	2.6
18	LS**R**FSWGAEGQ **R/Cit** PGFGYGG**R**	P02686.3	2.0
19	PGFGYGG **R/Cit** ASDY**K**SAH**K**GF**K**	P02686.3	0.6
20	LS**K**IF**K**LGG **R/Cit** DS**R**SGSPMA**R**	P02686.3	0.6
21	Y **R/Cit** VYCDMNTENGGWTVIQN**R**	P02675	0
22	MYLIQPDSSV**K**PY **R/Cit** VYCDM**R**	P02675	3.4
23	EAPSL **R/Cit** PAPPPISGGGY**R**AR	P02675	2.0
24	SI **R/Cit** YLQEIYNSNNQ**K**IVNL**K**	P02679.3	3.0

Results and Discussion

In situ digestion results were compared to the empirical data. For SP 10, prior to digestion only the synthetic peptide is detected and after digestion, peptides corresponding to PAPDR, LLLASPR and SCYK are detected, corresponding to a successful complete cleavage after 495_{Arg}. This is not the case after digestion of SP 10_{Cit}, where peptides corresponding to PAPDR and LLLASPCitSCYK are detected. All investigated peptides demonstrate this behavior.



Our results clearly demonstrate the inability of trypsin to cleave after citrulline residues. Hence, a miscleavage indicates the presence of the PTM. Furthermore, the shift in retention time between the citrulline and arginine peptides was large enough for 22 of the 24 peptides to ensure that coelusion is not occurring to a detectable extend, ensuring that both peptides can be identified.

Conclusion

- Our results clearly demonstrate the inability of trypsin to cleave after citrulline residues. As a result, a miscleavage can be used to distinguish a citrullination from a deamidation of asparagine or glutamine.
- The shift in retention time was, for 22 of 24 peptides large enough to ensure that both peptides could be identified.

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